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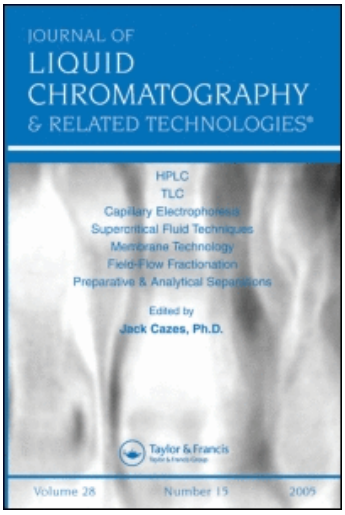

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CHIRAL RESOLUTION OF DIPEPTIDES BY LIGAND EXCHANGE CHROMATOGRAPHY ON CHEMICALLY BONDED CHIRAL PHASES

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ABSTRACT

This paper deals with studies on the optical resolution of glycyl-DL-amino acid dipeptides and diastereomeric dipeptides on three different chemically bonded chiral ligand exchange chromatography (LEC)-phases. The phases were prepared by binding L-proline or L-hydroxyproline to silica gel using different silanes as spacer. Using 10^{-5} M copper(II) sulfate as a mobile phase, eleven glycyl-dipeptides were resolved, nearly all of them with baseline separations. Several dipeptides containing two stereogenic centres were at least partially resolved into the four stereoisomers.

INTRODUCTION

Dipeptides are compounds of tremendous biological interest. Since the biological activity is mostly restricted to one of the enantiomers, the separation of the optical isomers has become increasingly important. Chiral separation of dipeptides is also of interest in protein research since certain peptide

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sequencing methods result in cleavage into dipeptide fragments. The optical purity control of the building blocks and the check for racemization processes in peptide synthesis are other important aspects.

Enantiomer separation of glycyl-DL-amino acid dipeptides (Gly-X-dipeptides) was done by LEC using chiral mobile phase additives[1] or chiral stationary phases[2] and by host-guest complexation using cyclodextrin-[3] or crown ether phases.[4,5] Furthermore, the use of TLC for the resolution of dipeptides has been described.[6] Several authors report the resolution of dipeptides containing two stereogenic centres into the two diastereomers; however, up to now only a few papers have described the chiral resolution of diastereomeric dipeptides into all four possible stereoisomers. Indirect separation of the stereoisomers of some dipeptides was carried out by Florence et al. using OPA and N-acetyl-L-cystein for chiral derivatization.[3] Oi et al. resolved some diastereomeric dipeptides by GC in the form of their N-TFA-isopropyl esters.[7] Hyun et al. resolved dipeptide methyl esters as their 3,5-dinitrobenzoyl derivatives using a chiral phase based on (S)-1-(6,7-dimethyl-1-naphthyl)isobutylamine.[8] The first direct resolution of an underivatized diastereomeric dipeptide into the 4 isomers has been reported by Gübitz,[2] using a chemically bonded chiral LEC-phase. Crownpack columns have been employed successfully for the optical resolution of a series of diastereomeric dipeptides by Hilton[4] and Esquivel et al.[5]

Recently, we succeeded in obtaining baseline resolutions of the stereoisomers of several diastereomeric dipeptides using (+)-18-crown-6-tetracarboxylic acid in capillary electrophoresis.[9]

This paper deals with comparative studies on three chemically bonded chiral LEC phases for the optical resolution of dipeptides.

EXPERIMENTAL

Chemicals And Materials

All reagents were of analytical grade. L-proline , L-hydroxyproline and copper(II) sulfate were obtained from Fluka (Buchs, Switzerland). 3-Glycidoxypropyltrimethoxysilane and 2-(3,4-epoxycyclohexyl)ethyltrimethoxysilane were purchased from Petrach Systems (Bristol, PA, USA), LiChrosorb 100, 5µm was obtained from Merck (Darmstadt, Germany). Dipeptides were from Bachem (Bubendorf, Switzerland) and from Sigma (Deisenhofen, Germany).

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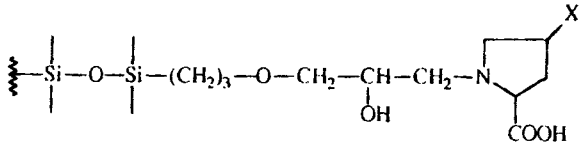
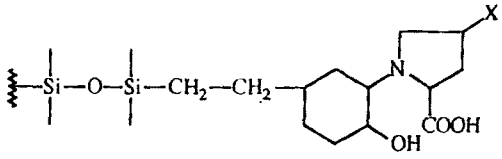


CSPIa: X=H
CSPIb: X=OH



CSPIIa: X=H
CSPIIb: X=OH

Figure 1. Chemical structure of the CSPs investigated

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Preparation of the CSPs:

CSP Ia and Ib (10): 4 g LiChrosorb 100, 5μm was suspended in 20 mL of toluene and after adding 2.4 mL of 3-glycidoxypropyltrimethoxysilane, the mixture was refluxed at 110°C with stirring for 6 h. The reflux condenser was kept at 65°C in order to remove the methanol formed in the reaction. The product was washed with toluene, methanol and acetone and dried at 50°C overnight. 4.4 g of either sodium prolinate (CSP Ia) or sodium hydroxyprolinate (CSP Ib) dissolved in 40 mL of methanol were shaken with the product for 48 h at room temperature. The modified silica was washed with methanol and loaded with copper(II) ions by shaking with a 15% solution of copper(II) sulfate.

CSP IIa and IIb were prepared analogously, but 2-(3,4-epoxycyclohexyl) ethyltrimethoxysilane was used instead of 3-glycidoxypropyltrimethoxysilane.[11]

Elemental analysis: CSP Ib: C 9.2, H 1.4, N 0.7%
CSP IIa: C 9.0, H 1.4, N 0.6%
CSP IIb: C 9.5, H 1.6, N 0.6%

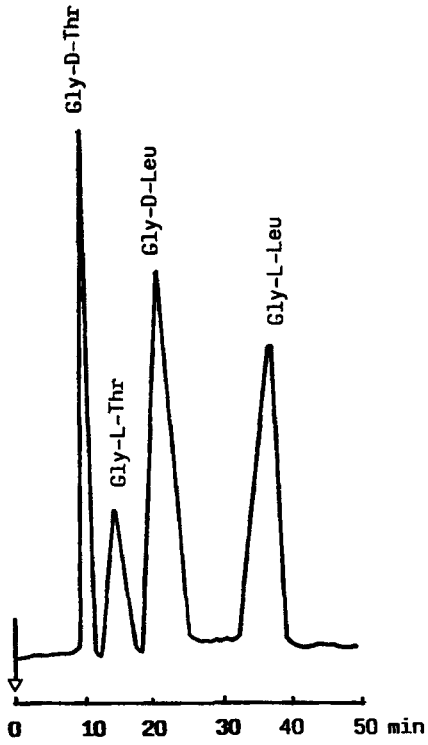

Figure 2. Chiral resolution of Gly-DL-Thr and Gly-DL-Leu on CSP IIb. Experimental conditions: Mobile phase: 10^{-5} M Cu(II), flow: 1 mL/min, Temp.: 50°C, detection: UV, 223 nm

HPLC Conditions

HPLC was performed using a Merck-Hitachi L-6200A intelligent pump and an L 4250 UV/VIS detector. Samples were injected by a Rheodyne Model 7161 six-port valve equipped with a 20 µl loop. The chiral phases were packed into stainless-steel columns (250 x 4.6 mm) by the descending slurry technique in methanol. As mobile phase a 10^{-5} M copper(II) sulfate solution was used in all cases.

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Table 1

Chiral Resolution of Gly-X-Dipeptides on CSP Ib, IIa and CSP IIb

	Ib			IIa			IIb		
	k'_D	k'_L	α	k'_D	k'_L	α	k'_D	k'_L	α
Gly-DL-Ala	1.13	1.50	1.33	1.40	1.60	1.14	2.09	2.76	1.32
Gly-DL-Nval	1.94	3.00	1.55	n.d.	n.d.	n.d.	4.18	7.41	1.77
Gly-DL-Nleu	2.00	3.13	1.57	3.67	6.00	1.63	5.61	12.25	2.18
Gly-DL-Val	2.63	4.25	1.62	3.83	5.67	1.48	1.74	2.81	1.62
Gly-DL-Leu	3.00	5.00	1.67	5.30	8.17	1.54	5.19	10.32	1.99
Gly-DL-Phe	1.50	4.00	2.67	2.66	5.00	1.87	3.19	7.11	2.23
Gly-DL-Trp	2.19	8.13	3.71	n.d.	n.d.	n.d.	2.01	4.31	2.40
Gly-DL-Met	1.44	2.19	1.52	2.17	3.17	1.46	3.27	6.25	1.91
Gly-DL-Ser	0.75	1.00	1.33	1.50	1.96	1.31	0.71	0.71	1.00
Gly-DL-Thr	1.25	2.00	1.60	2.00	2.50	1.25	2.03	3.51	1.73
Gly-DL-Asn	0.94	1.25	1.33	1.10	1.10	1.00	1.53	2.12	1.39

Experimental conditions: Mobile phase: 10^{-5} M Cu(II), flow: 1mL/min, Temp.: 50°C, detection: UV, 223 nm, (n.d. = not determined).

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RESULTS AND DISCUSSION

The surface coverage was comparable for all the three phases investigated. Based on the results of the elemental analysis the surface coverage was found to be about 2 μmol/m^2 on average for the different phases. Two different epoxyfunctional silanes, 3-glycidoxypropyltrimethoxysilane and 2-(3,4-epoxycyclohexyl)ethyltrimethoxysilane, were used as spacer (Fig.1). The cyclic moiety in the spacer of CSP II represents a more rigid structure element close to the chiral centre, which is expected to enhance stereoselectivity. CSP IIa containing L-proline as chiral selector ligand, has been shown to exhibit improved enantioselectivity for amino acids and hydroxy acids.[11] For the resolution of dipeptides, L-hydroxyproline as selector ligand was found to be superior to L-proline. The proline phase Ia showed almost no enantioselectivity for dipeptides. These results are in agreement with observations of Florence et al.,[3] who did not succeed in resolving dipeptides on Chirapack WH, which has identical structure with our CSP Ia. In the case of the phases containing the cyclic spacer, both the L-proline- (IIa) and L-hydroxyproline phase (IIb) resolved dipeptides; however, CSP IIb showed significantly higher α-values.

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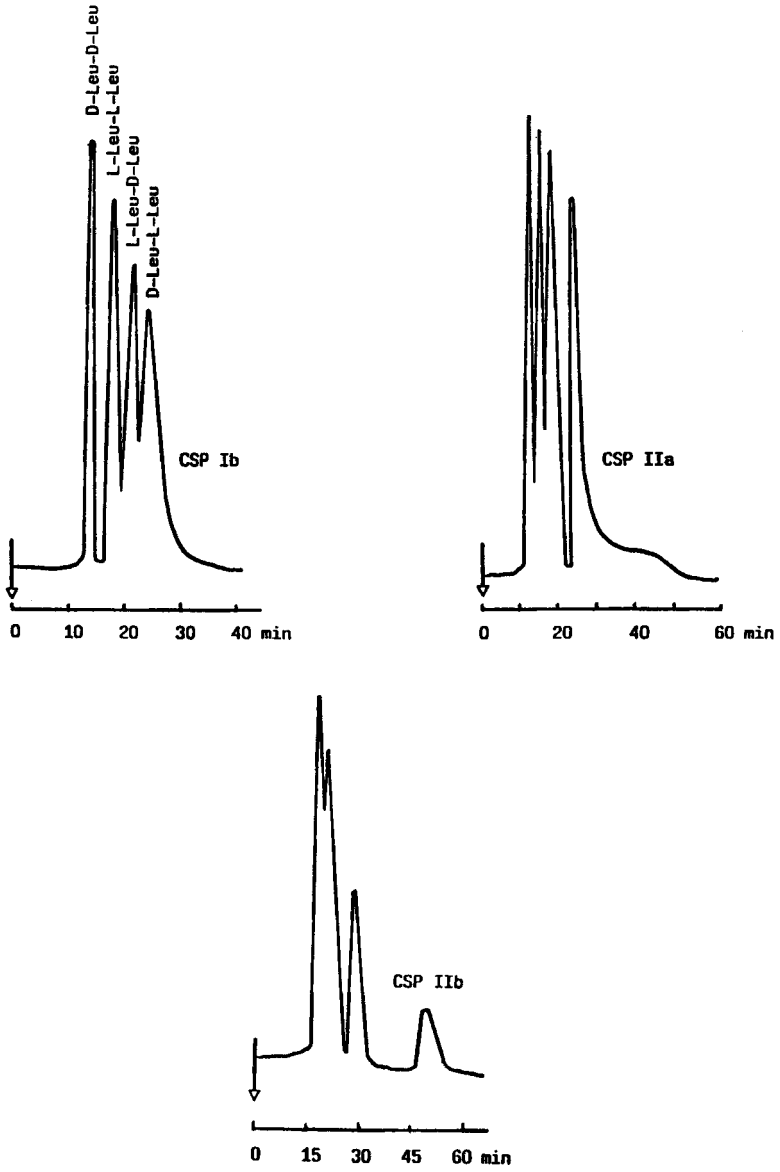

Figure 3. Chiral resolution of DL-Leu-DL-Leu on CSP Ib, IIa and IIb. Experimental conditions as in Fig.2

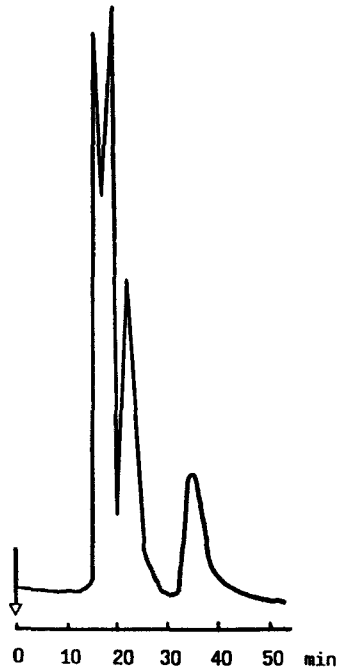

Figure 4. Chiral resolution of DL-Leu-DL-Val on CSP Ib. Experimental conditions as in Fig.2

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While the pH optimum was found to be 4.5 for the resolution of amino acids,[10-12] resolution of dipeptides occurred only at pH 7. A 10^{-5} M copper(II) sulfate solution without any buffer turned out to be optimal. Raising the temperature to 50°C resulted in significant improvement in resolution.

Table 1 shows a comparison of the capacity factors and α-values for Gly-X-dipeptides on CSP Ib, IIa and IIb. CSP IIb showed the highest separation factors. Gly-X-dipeptides containing bulky and hydrophobic substituents were much better resolved than those with polar substituents. Nearly all Gly-X-dipeptides investigated showed baseline separation. The elution order was found to be D before L in all cases indicating a stronger complexation of the L-enantiomer with the chiral selector. The same elution order was observed for amino acids.[10-12] Fig.2 shows the resolution of Gly-DL-Thr and Gly-DL-Leu on CSP IIb.

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Table 2

Chiral Resolution of Diastereomeric Dipeptides on CSP Ib, CSP IIa and CSP IIb

	CSP Ib				CSP IIa				CSP IIb			
	k'_1	k'_2	k'_3	k'_4	k'_1	k'_2	k'_3	k'_4	k'_1	k'_2	k'_3	k'_4
DL-Leu-DL-Leu	0.78	1.00	1.39	3.22	5.0	5.7	9.7	16.7	2.56	3.67	4.62	5.54
DL-Leu-DL-Val	0.78	1.00	1.56	2.94	1.7	1.7	3.8	6.4	2.36	2.64	3.10	3.89
DL-Ala-DL-Ser	0.11	0.11	0.18	0.18	0.79	0.79	0.79	0.79	0.68	0.68	0.68	0.68
DL-Leu-DL-Ala	0.60	0164	0.73	1.13	1.77	1.77	2.26	3.01	1.29	1.29	1.50	2.15
DL-Leu-DL-Phe	0.55	0.89	1.00	1.78	4.53	4.53	8.16	12.56	3.62	3.62	5.50	7.73
DL-Ala-DL-Val	0.55	0.69	0.82	1.78	2.84	2.84	3.33	6.60	1.62	1.62	2.07	3.01
DL-Leu-DL-Tyr	n.d.	n.d.	n.d.	n.d.	2.7	2.7	5.8	8.0	n.d.	n.d.	n.d.	n.d.

Experimental conditions as in Table 1, (n.d. = not determined).

Partial resolution was also obtained with dipeptides containing 2 stereogenic centres. In this case CSP Ib showed the best results (Table 2). Six diastereomeric dipeptides were at least partially resolved into the four possible stereoisomers. Fig. 3 shows a comparison of the resolution of DL-Leu-DL-Leu on the three phases investigated. The elution order was DD, LL, LD and DL on all phases. Different elution profiles were observed on the different phases. While on CSP Ib the enantiomeric pairs DD and LL showed baseline resolution, on CSP IIa and IIb the enantiomeric pairs LD and DL were baseline resolved. The other peaks overlapped partially. The differing peak size of the isomers is due to abnormal isomer composition, which varies among different commercial products. Similar observations have been reported by Esquivel et al.[5] The elution order was determined for DL-Leu-DL-Leu only since in the case of the other diastereomeric dipeptides the individual stereoisomers were not available. Fig.4 shows the resolution of DL-Leu-DL-Val on CSP Ib.

Studies on the structure of the mixed complex between the selector ligand and the dipeptides are in progress.

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